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Temperature-Induced Fluorescence Changes[1]

A SCREENING METHOD FOR FROST TOLERANCE OF POTATO (*SOLANUM* SP.)

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ABSTRACT

Field-grown tuber-bearing potatoes were screened for frost tolerance in a late stage of development. Three different clones of *Solanum tuberosum* L. and two interspecific crosses between clones of *S. tuberosum* and the wild potato species *S. demissum* Lindl. were studied. Two different methods were used. (a) Temperature-induced fluorescence changes of intact leaves were measured in freeze-thaw cycles between 20°C and −10°C. The variable fluorescence pattern was characterized in relation to frost tolerance. (b) Controlled freezings of plants in a climate chamber with successively increased low temperature stress, of 1 to 2 hours duration during the dark period. Freezing damages were classified visually.

The short-term frost during the fluorescence measurement was compared with the long-term frost treatments in the climate chamber. The results of the two were identical to ranking of the different clones for frost tolerance. The temperature-induced fluorescence changes also monitored progressive damages to the chloroplast membranes when plants were exposed to successively lower temperatures in a controlled climate chamber freezing test. It was deduced from the fluorescence measurements that the freezing injury of potato occurs on the water splitting side of photosystem II.

In northern Sweden, frost is a problem during the last month before the crop *Solanum tuberosum* reaches maturity. Taxonomically, *S. tuberosum* is placed in the group identified as the most frost sensitive tuber bearing *Solanum* species. These are considered unable to cold harden (6).

Membrane damage is a universal manifestation of freezing in biological systems and is commonly inferred to be the primary cause of injury. Increased frost tolerance deals with the problem of modifying the membrane system (3) or the surroundings of the susceptible membranes (11). Osmotic pressure, water content, lipids, proteins, pigments, starch, and sugar content are of primary interest in studies of frost tolerance (5). It is also known that fertilization (K^+) and growth substances (ABA, CCC[2]) can influence frost tolerance in potatoes (5, 7).

Controlled freezing in climate chambers is a frequently employed method of studying frost tolerance. Several simple frost tolerance tests have evolved (2). The excised leaflet test (12) measures the leakage of ions from leaves which are frozen and thawed. Li *et al.* (6) described other test procedures. A new approach using changes in Chl fluorescence to follow the development of chilling injury has been outlined by Smillie (10).

The measurement of fluorescence gives unique possibilities to study environmental effects on the chloroplast thylakoid membrane system (14). Temperature induced (20°C to −20°C) fluorescence changes have proved to be a powerful tool for measuring the functional properties of the chloroplast thylakoids both in isolated chloroplasts and intact leaves (13–15) (E. Sundbom and G. Öquist, unpublished data). It should be emphasized that this does not automatically infer that the chloroplast thylakoids are the primary site of freezing injury. However, chloroplast thylakoids are the most well characterized membranes in the plant cell and the Chl molecules interact as an intrinsic fluorescent probe of the thylakoid membranes and its surroundings (8). In this study, temperature-induced Chl fluorescence is applied to screen for frost tolerance of different clones of *S. tuberosum* and of interspecific crosses between *S. tuberosum* and *S. demissum*.

MATERIALS AND METHODS

Plant Materials and Cultivation. Three different clones of *S. tuberosum* L., designed No. 1, 2 and 3, and two interspecific crosses between clones of *S. tuberosum* and the wild potato species *S. demissum* Lindl., designed Nos. 4 and 5, were grown at Svalöf Seeds Ltd, North Swedish Department, Röbäcksdalen, Umeå. The clones were specified in detail by Sundbom (13).

Tubers were planted separately in pots with soil (pot volume 5 $\times 10^{-3}$ m^{-3}) in the middle of June. The plants were fertilized with 4 g NPK, 8-7-16 (Supra AB Förenade superfosfatfabriker, Sweden) per pot. In the middle of August the plants were divided into two equal groups one of which was placed immediately into a climate chamber. The other was placed in the chamber 14 d later. The climate conditions were as follows: photoperiod, 17 h; temperature, 15°C in light and 6°C in dark; RH 70% to 90%; quantum flux density 230 to 240 μE m^{-2} s^{-1}. The light source was metal halogen lamps, Osram HQl-T 400 w/DH. The quantum flux density was measured with a quantum meter (Lambda Li-185 A, quantum sensor; Lincoln, NE).

Controlled Freezing in Climate Chamber. In the dark, a subzero temperature period of 0.5 to 2 h duration was inserted with minimum temperatures of −0.5°C, −1.5°C, −3.0°C, respectively (Table I). A cooling rate of 6°C/h and a warming rate of 5°C/h of the climate chamber was used when the subzero periods were introduced and terminated, respectively. The end of the low temperature period coincided with the end of dark period. The low temperature treatment of the plants was introduced in two different ways. (a) Plants of the five clones were stepwise passed through successively decreasing low temperature regimes by using frost regimes a to d until all clones were frost killed (Table I). The following scheme was followed: regime a, 6 d; regime b, 2 d; regime c, 4 d; and regime d, 6 d. (b) Plants of the five different clones were directly exposed to regime e, for 8 d. The numbers of frozen stems and the percentages of frozen leaves of each clone

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[2] Abbreviations: CCC, chlorocholine chloride; F_s, steady-state fluorescence; F_o, ground fluorescence; F_m, maximum fluorescence; Q (Q^-), electron acceptor of PSII and its reduced form; $P680^+$, oxidized reaction center chlorophyll of PSII.

were observed directly every day.

Fluorescence Measurements. Temperature-induced fluorescence changes were measured from intact fully expanded excised leaves. The cooling rate was 1°C/min and the quantum flux density of the exciting light (400–500 nm) was 2 $\mu E\ m^{-2}\ s^{-1}$. The circular irradiated leaf area and hence, the area from which fluorescence emission was detected was 176 mm^2. The fluorescence emission was modulated by a chopper placed in front of the detector, a photomultiplier tube (RCA C 31026). The photomultiplier signal was fed into a lock-in amplifier (Princeton Applied Research, New Jersey, model 124 together with model 116 differential preamplifier).

The evaluation of the fluorescence data was made in two ways. The respective data evaluation was applied to fluorescence measured at above-freezing temperatures of the leaf and at subfreezing temperatures of the leaf.

Above Freezing. The temperature-induced fluorescence change (ΔF_s) was compared with the initial fluorescence level ($F_s = F_o$) in the temperature range between 20°C and the freezing point of the leaf. The results from this data treatment are classified into the following three groups: 1, increase; 0, invariable; −1, decrease.

Subfreezing. The relative intensity of the maximum steady-state fluorescence (F_m) evolved in the temperature range between the freezing point of the leaf and −10°C, was compared to the initial fluorescence level (F_o). The different clones were compared and ranked according to the F_m values, from highest (rank 5) to the lowest (rank 1).

The temperature of the leaves was determined by a Cu-constantan thermocouple placed in the fluorescence cuvette attached to the upper surface of the leaf. The thermocouple, and the photomultiplier signal, was recorded on a strip chart 2-pen recorder (Tekman 220/2; Tekman Electronics Ltd., England). As a constant cooling rate of 1°C/min of the leaf was maintained, the probability of extracellular freezing of water was very high. At the moment when ice crystalization occurred, heat was given off and the temperature transiently increased and an exotherm was registered. The lowest point of the temperature curve registered immediately prior to the onset of the exotherm was taken as the freezing temperature of the leaf.

RESULTS

Controlled Freezing in Climate Chamber. The percentage of frost injured leaves of clones 1–5 was determined by direct observation after 1 d of low temperature treatment with regime e, (−3°C). After one low temperature period, clone No. 3 was totally frost killed and the freezing injury was pronounced in clones No. 1 and 5, whereas only one-third of the leaves of No. 4 were affected and clone No. 2 remained unaffected. After 3 d of the frost treatment, clones No. 2 and No. 4 appeared to be equally injured. Clone No. 4 was, after 8 d, somewhat less affected than clone No. 2. Identical results were obtained irrespective of whether the plants were transferred from the field to the climate chamber test in the middle or in the end of August.

When the plants were exposed to successively lower temperatures by using frost regimes a to d (Table I), all plants except clone No. 3 remained unaffected throughout regimes a, b, and c. All plants showed frost injuries when transferred to regime d (−3°C). Clone No. 3 could only withstand regime c (−1.5°C) for 3 d. On day 4 of exposure to regime c, clone No. 3 exhibited 100% mortality.

Table I. *Frost Regimes during Dark Used for Controlled Freezings in Climate Chamber of 15 Hours Light, 6 Hours Dark*

Frost Regime	Temp	Duration	Subzero Temp Each Day
	°C	h	
a	−0.5	0.5	2
b	−1.5	1	2
c	−1.5	2	3
d	−3.0	2	4
e	−3.0	1	3

The freezing temperatures (*cf.* "Materials and Methods") of different clones are presented in Table II. The differences in freezing temperatures were small and cannot easily explain the differences observed from the controlled freezing experiment in the climate chamber. There was no obvious correlation between water content of the leaves and sensitivity to low temperatures among the clones tested (Table II).

Temperature-Induced Fluorescence Changes of Intact Leaves. Figure 1 represents a typical fluorescence pattern of leaves from clone No. 2 (upper curve equals control plants). Note that the inducible fluorescence changes were lost after a single freeze-thaw cycle, between 20°C and −10°C. This loss of inducible fluorescence changes was observed even if the subfreezing temperature was maintained only 1 min.

An increase of fluorescence at above-freezing temperatures and the highest F_m values were observed for clones No. 2 and No. 4. In the climate chamber tests, these clones were also shown to be the most frost resistant. The most sensitive clone, No. 3, showed a decrease of fluorescence at above-freezing temperatures and the lowest F_m values at subfreezing temperatures measured. Fluorescence patterns from clones No. 1 and No. 5 exhibited intermediate responses. The fluorescence from these clones was invariable at above-freezing temperatures and intermediate F_m values, compared to the other clones was measured at subfreezing temperatures.

A close examination of the fluorescence responses of clone No. 2 from repeated low temperature treatments in the climate chamber (regime e) during day 1, 2, and 3 is presented in Figure 1. It is obvious that both the inducible fluorescence and the fluorescence maximum decreases with prolonged freezing-stress. The magnitude of these changes may differ slightly between different leaves of the plants. However, none of the leaves were unaffected after the first day even though no visible injuries were detectable. At the end of the freezing-experiment on day 8, all clones were severely freeze-damaged. Hence, the inducible fluorescence changes were absent.

DISCUSSION

Controlled freezings in climate chambers and temperature-induced fluorescence changes are identical in their ranking of the different clones for frost susceptibility. Thus, it appears that the Chl fluorescence method can be used to rank the tolerances to low temperatures on different and closely related clones of *S. tuberosum.* This is in accordance with the results shown for chilling sensitive species by Smillie (10) by using a fluorescence induction

Table II. *Freezing Temperatures of Leaves of Different Clones of Solanum sp.*

Mean ± SE, n = 4–20. Dry wt in % of fresh wt of leaves from the different clones, taken in from the field on August 15 and 29, respectively. Mean ± SE, n = 4.

Clone No.	n	Freezing Temp	Dry Wt in % of Fresh Wt	
			15 August	29 August
		°C		
1	16	−2.9 ± 0.3	10.13 ± 0.03	10.03 ± 0.03
2	20	−2.5 ± 0.2	10.65 ± 0.03	10.27 ± 0.02
3	4	−2.1 ± 0.4	12.81 ± 0.00	12.19 ± 0.02
4	7	−2.2 ± 0.2	9.66 ± 0.01	9.86 ± 0.04
5	12	−2.3 ± 0.3	10.20 ± 0.02	10.07 ± 0.04

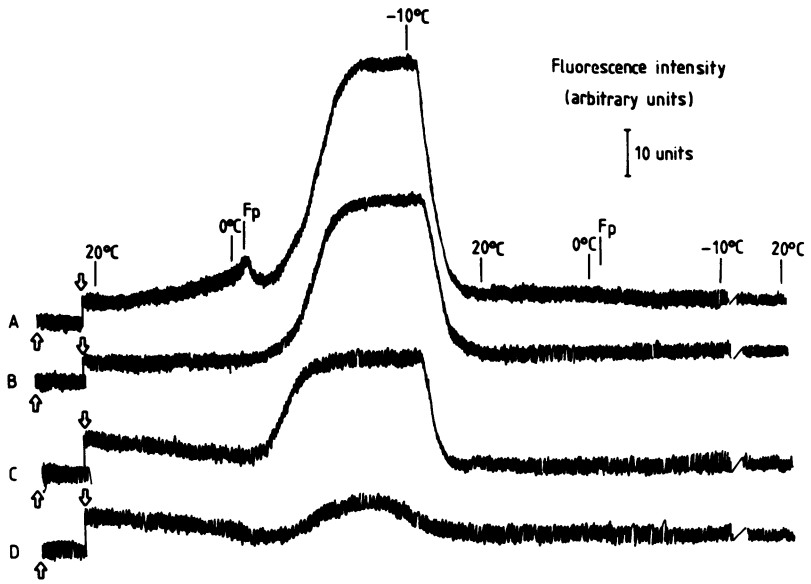

FIG. 1. Temperature-induced fluorescence changes of leaves of clone No. 2, between 20°C and −10°C. The cooling and warming rate of the two repeated freeze-thaw cycles was 1°C/min. A, control plants; B, 1 d of frost regime e; C, 2 d of frost regime e; D, 3 d of frost regime e; Fp, freezing point of the leaf; ↑, excitating light off; ↓, excitating light on.

assay. The Chl fluorescence method also monitors the progressive damage to chloroplast membranes, either directly or indirectly, caused by freezing injury not detectable in the appearance of the leaves. This is shown for clone No. 2 which was treated in the climate chamber without any visible injury (Fig. 1). After 1 d at low temperature, the inducible fluorescence changes of both above- and subfreezing temperatures were altered and showed similar and corresponding values to the more frost sensitive clones. After two (or more) repetitions of the low temperature treatment of clone No. 2, the inducible fluorescence changes further decreased.

The temperature induced fluorescence changes were studied in weak exciting light (*cf.* "Materials and Methods"). Hence, F_o fluorescence was induced at the onset of light (20°C). The fluorescence *versus* the quantum flux densities of the exciting light relate linearly when measured for whole leaves (0.1–30 μE m^{-2} s^{-1}, data not shown). When the temperature was lowered to subfreezing temperatures the reoxidation of the electron acceptor Q of PSII by secondary electron acceptors of the electron transport chain between the two photosystems becomes inhibited (9, 16). This results in maximum steady-state fluorescence (F_m) since Q under these conditions remained reduced in continuous exciting light. The fluorescence method used here thus monitors the fluorescence increase as PSII becomes inhibited on the reducing side. However, if a damage occurs on the water splitting side of PSII there will be an accumulation of $P680^+Q^-$ which is an effective quencher of variable fluorescence (1). In the extreme case of total inhibition on the water splitting side of PSII temperature-induced variable fluorescence changes will not be detected. Thus, factors inhibiting on the oxidizing side of PSII are expected to decrease the rise in fluorescence above F_o; on the contrary, factors affecting the reducing side of PSII are expected to increase fluorescence. The fluorescence pattern observed with the *Solanum* clones indicates a primary effect of the freezing injury to the oxidizing side of PSII. A similar conclusion was made by Smillie (10), from studies of fluorescence kinetics of tomato-potato hybrids at 0°C.

CONCLUSIONS

The measurement of temperature-induced fluorescence changes of *S. tuberosum* leaves seem to be a very sensitive method for screening of large number of samples in the ranking of plants for frost tolerance. The introduction of low temperature stress of short duration to induce fluorescence changes and, hence, to inhibit photosynthetic electron transport seem to simulate long-term subzero effects on the plant. In addition, the method also monitors progressive damages to the chloroplast membranes. Clones No. 4, and 2 were classified as the most frost resistant, and clone No. 5 was classified as an intermediate resistant clone. Both clones No. 4 and No. 5 are interspecific crosses between different clones of *S. tuberosum* and the wild potato species *S. demissum*. *S. demissum* has been reported with respect to frost tolerance as resistant to temperatures of −4°C to −5°C (7). In addition, Kovalenko (4) tested hybrides of *S. tuberosum* (european cultivars) and *S. demissum*, and found that these hybrides always had higher frost resistance than the *S. tuberosum* cultivars.

Our opinion is that the described Chl fluorescence method is useful in screening for frost tolerance and might in breeding experiments, serve as a method for a better understanding of the photosynthetically related physiology inherent in processes of acclimation in adaptation to environmental temperature stress.

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